

Figure S3. Knockdown of cilia proteins and RNA-Seq comparison of the transcriptomes of PC12 cells transfected with siRNAs targeting IFT88 or non-targeting control siRNAs. (A) Immunoblot of total cell lysates from PC12 cells transfected with siRNAs targeting IFT88 or non-targeting control siRNAs (Con). Cell lysates were generated 48 hours after transfection and immunoblots probed with an anti-IFT88 antibody and anti-β-actin as a loading control. (B) Densitometric analyses were performed and mean relative IFT88 protein levels calculated (n=6). Data were normalised to β-actin. (C) Relative RNA expression of Cep164 in PC12 cells 48 hours after they were transfected with siRNAs targeting this transcript. Cep164 levels are expressed relative to those in cells transfected with a non-targeting control siRNAs (arbitrary units). Data was normalized to levels of peptidylpropyl isomerase A. (D, E) Quantification of axoneme length from PC12 cells transfected with siRNAs targeting IFT88 (D) or Cep164 (E), compared to cells transfected with non-targeting control siRNAs. 48 hours after transfection. Error bars indicate 2x SEM. Statistical tests: t-test. * P<0.05, **P<0.01, ****P<0.001. (F) Principal component analysis (PCA) of RNA-seq expression data from IFT88 knockdown (R10-R12) and control cells (R13-R15; n=3 per condition). (G) Volcano plot showing log10 FDR-adjusted q-values versus log2 fold change between IFT88 knockdown and control cells. The vertical and horizontal dotted lines indicate 2x or -2x fold change and P = 0.01, respectively.